Atomic Resolution Study of the Effect of pH on Cholesterol Oxidase

P. Lario (McGill U.) and A.Vrielink (U. California at Santa Cruz)

Abstract No. Lari3292
Beamline(s): **X8C**

Introduction: Cholesterol oxidase is a 55 KDa, bi-functional enzyme that catalyses the oxidation and isomerization of 3β-hydroxy-steroids having a double bond at Δ5-Δ6 of the steroid backbone. It contains a cofactor, FAD non-covalently bound to the enzyme. Reduction of the FAD occurs reversibly by two one-electron steps or by a two-electron step.[1] The pyrimidine ring of the isoalloxazine moiety is able to take on additional electrons and hence groups that can lower its negative charge density will increase the redox potential of the flavin. In addition, the environment of the protein will alter the stability of the intermediate radical forms.

Methods and Materials: The crystals of cholesterol oxidase from Streptomyces have been grown in a wide pH range, from 4 – 9. Atomic resolution data has been collected for five crystals grown at varying pH's and should enable us to estimate the pKa values for the different catalytic residues and the FAD cofactor. Precise coordinates for this prosthetic group as well as the surrounding protein environment will aid in our understanding of the redox reactivity of this group. Knowledge of the position of the hydrogen atoms in the active site of cholesterol oxidase will provide new structural information about the enzyme mechanism.

Results: Atomic resolution data for cholesterol oxidase has been collected and processed at the following pH's; 4 (0.95 Å), 5.2 (0.95 Å), 6(1.05 Å), 7.5(0.91 Å), 9(0.91 Å). The pH 5.2 crystal has been refined to a final R of 9.9 % and R_{free} of 12.1 %. Shown in **Figure 1** is an example of the electron density around the isoalloxazine moety of the flavin. We are currently in the process of refining the other pH data sets.

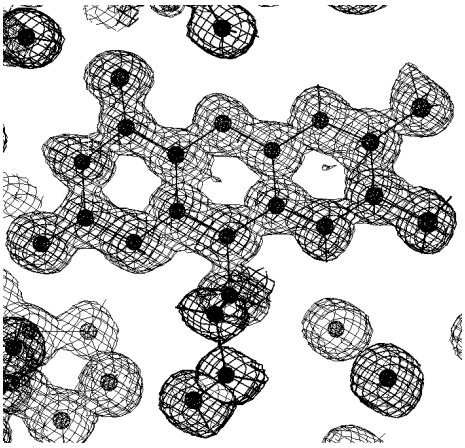

Figure 1. Electron density around the isoalloxazine moiety of the FAD cofactor. The map is contoured at 1 σ level.

Conclusions: From the refined structures a pH titration curve will be generated for all acidic and basic residues and thus an estimation of their pKa values will be obtained. The atomic resolution maps reveal much more detail and allow one to model diffuse and/or low occupancy waters, locate hydrogen atoms and refine the occupancy of the multiple conformations observed for some residues. This provides a better analysis of the hydrogen-bonding network within the water lattice of the active site and how the pH modulates the environment around the FAD cofactor.

Acknowledgments: This work was financially supported by a research grant from the Medical Research Council of Canada

References: 1. Ghilsa, S., and Massey, V. (1989) *Eur. J. Biochem*. **181**, 1-17